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(54) (TITLE OF THE INVENTION) Organ transplant rejection suppressant

(57) (ABSTRACT)
(PROBLEM) To provide a drug for suppressing organ transplant rejection
(MEANS FOR SOLVING) An organ transplant rejection suppressant containing P-selectin binding inhibitor. For example, said suppressant containing anti-P-selectin antibodies as the P-selectin binding inhibitor.

(SCOPE OF PATENT CLAIMS)
(CLAIM 1) An organ transplant rejection suppressant having a P-selectin binding inhibitor as an active ingredient.
(CLAIM 2) The suppressant as described in Claim 1, wherein the P-selectin binding inhibitor is an anti-P-selectin antibody, a P-selectin ligand or derivative thereof, a P-selectin ligand specific antibody, P-selectin or fragments thereof, a biosynthesis inhibitor of P-selectin ligands, or a P-selectin expression inhibitor.
(CLAIM 3) The suppressant as described in Claim 1, wherein the P-selectin binding inhibitor is an anti-P-selectin antibody.
(CLAIM 4) The suppressant as described in any one of Claims 1 through 3, wherein the organ transplant is a heart transplant.
(DETAILED DESCRIPTION OF THE INVENTION)
(0001)
(TECHNICAL FIELD OF THE INVENTION) The present invention relates to a novel medical application for P-selectin binding inhibitors. More specifically, the present invention relates to organ transplant rejection suppressants having P-selectin binding inhibitors as an active ingredient.
(0002)
(PRIOR ART) When an organ becomes dysfunctional due to various serious diseases, transplantation of an organ from another human is conducted as treatment. Recently, attempts to transplant animal organs into humans have also started. In transplantation of organs to humans, the greatest problem clinically is rejection. This is when the transplanted organ (graft) fails because it is not successfully grafted into the body of the person receiving the transplant (the recipient). The first conceivable cause of rejection is allotransplantation immune reaction. When a substance other than the organism's own components invade an organism, the organism recognizes that substance as an antigen, and as an element of its self-defense, tries to eliminate the foreign matter through cellular immunity based on lymphocytes and through humoral immunity based on produced antibodies. In transplant rejection, it is thought that the recipient recognizes the graft as an antigen, and the rejection takes place through T-cell based immune response which exhibits a cascade of T-cell activation and proliferation and graft destruction. Therefore, a group of drugs called immunosuppressants are used to suppress this reaction during organ transplantation. Such drugs include corticosteroids (prednisolone) and cyclosporin A, and recently, FK-506 has also come to be commonly used. However, these drugs are not completely effective, and side effects have also been reported. A second conceivable cause is ischemic reperfusion injury. In organ transplantation, when the graft is extirpated, the blood flow to it is temporarily cut off (ischemia), after which blood flow is restarted (reperfusion) upon transplantation into the recipient. It is well known that the operation of ischemia-reperfusion causes injury to the tissue at the reperfusion site, at least one possible mechanism of which may involve the activation of neutrophils contained in the blood. Namely, it has been demonstrated in animal experiments that reperfusion injury is greatly reduced in animals in which neutrophils have been reduced. Therefore, suppressing the activation of white blood cells by some method is thought to be an effective means for preventing ischemic reperfusion injury and preventing organ damage during organ transplantation.
(0003) White blood cells have the function of disposing of foreign matter that has penetrated from outside the organism, and normally perform elimination of foreign matter. Furthermore, when tissues sustain trauma, white blood cells gather at the trauma site and contribute to the repair of the tissue. Generally, inflammatory reaction becomes a problem when the self-defense by these white blood cells works excessively and ends up damaging the organism's own tissues. White blood cells that have gathered at an inflammation site undergo activation and work to break down and dispose of foreign matter by releasing the protease stored in cells and by producing active enzymes. A marked accumulation of white blood cells often occurs after transplantation in the tissue surrounding the organ transplantation site. This means that tissue damage is occurring at the white blood cell accumulation site. While various attempts have been made to treat this, there are currently no effective means.
(0004)
(PROBLEM TO BE SOLVED BY THE INVENTION) The object of the present invention is to find a drug which will prevent the rejection of organ transplants by preventing the accumulation of white blood cells which occurs during organ transplantation, and their subsequent activation.
(0005)
(MEANS FOR SOLVING THE PROBLEM) The inventors, as a result of concerted research on alleviating this sort of rejection that takes place during organ transplantation, found that, in such pathologies in animals, the interaction between white blood cells and vascular endothelial cells plays an important role. The inventors furthermore ascertained, through investigation using antibodies, that this pathology is controlled by P-selectin, which contributes to the adhesion of white blood cells to vascular endothelial cells, thereby completing the present invention.
(0006) Namely, the present invention is
(1) An organ transplant rejection suppressant having a P-selectin binding inhibitor as an active ingredient.
(2) The suppressant as described in (1) above, wherein the P-selectin binding inhibitor is an anti-P-selectin antibody, a P-selectin ligand or derivative thereof, a P-selectin ligand specific antibody, P-selectin or fragments thereof, a biosynthesis inhibitor of P-selectin ligands, or a P-selectin expression inhibitor.
(3) The suppressant as described in (1) above, wherein the P-selectin binding inhibitor is an anti-P-selectin antibody.
(4) The suppressant as described in any one of (1) through (3) above, wherein the organ transplant is a heart transplant.
(0007) The present inventors discovered that when the heart of another rat was transplanted into rats, the rejection of the transplanted heart was clearly suppressed by anti-P-selectin antibodies. This fact indicates that P-selectin mediates the interaction between white blood cells and vascular endothelial cells and plays an important role in the activation of white blood cells and damage to tissues surrounding the ischemic reperfusion site, and that P-selectin binding inhibitors are suited for ameliorating the organ transplant rejection that accompanies these factors.

(0008) "P-selectin binding inhibitor" refers to a substance which inhibits binding between P-selectin in vascular endothelial cells and ligands in white blood cells. The P-selectin binding inhibitors in the present invention include for instance the following four types, based on manner of inhibition.

① Substances which inhibit binding with ligands on white blood cells by binding to P-selectin. Examples of inhibitors of this type include anti-P-selectin antibodies, P-selectin ligands and derivatives thereof.

② Substances which inhibit P-selectin binding by binding to ligands on white blood cells. Examples of inhibitors of this type include P-selectin ligand specific antibodies, P-selectin and fragments thereof.

③ P-selectin ligand biosynthesis inhibitors

④ P-selectin expression inhibitors

Particularly preferable among these are substances which inhibit binding with ligands on white blood cells by binding to P-selectin, and P-selectin expression inhibitors.

(0009) Anti-P-selectin antibody signifies immunoglobulin which recognizes P-selectin, selectively binds to P-selectin, and thereby suppresses intracellular adhesion. Such antibodies can be polyclonal or monoclonal. The origin of these antibodies is not restricted; examples include antibodies of mouse or human origin, chimeric antibodies combining portions of human and mouse antibodies, anthropomorphic antibodies, etc. Specific examples include PB1.3 as described in WO93/21956.

(0010) P-selectin ligands and derivatives thereof include surface glycoproteins and glycolipids of white blood cells, etc. and oligosaccharides which are the terminal structures thereof, as well as their derivatives. Examples of the oligosaccharides and their derivatives include sialyl Lewis x and sialyl Lewis x derivatives, Lewis x and Lewis x derivatives, sialyl Lewis a and sialyl Lewis a derivatives, Lewis a and Lewis a derivatives, sulfated saccharides, phosphated saccharides, sulfatides, and the like (e.g. Varki et al., Proc. Natl. Acad. Sci. USA 91, 7390 (1994); WO94/26760). Examples of glycoproteins include PSGL-1 (e.g. Sako et al., Cell 75, 1179 (1993)) and the like.

(0011) P-selectin ligand specific antibody signifies an antibody specific to the aforementioned ligands. Such antibodies can be either monoclonal or polyclonal. The origin of these antibodies is not restricted; examples include antibodies of mouse or human origin, chimeric antibodies combining portions of human and mouse antibodies, anthropomorphic antibodies, etc. Specific examples include anti-sialyl Lewis x antibodies, anti-sialyl Lewis a antibodies, anti Lewis x antibodies, anti Lewis a antibodies and the like (e.g. Fukushima et al., Cancer Res. 44, 5279 (1984); Shitara et al., Cancer Res. 47, 1267 (1987); and Takada et al., Biochem. Biophys. Res. Commun. 179, 713 (1991)).

(0012) P-selectin and fragments thereof signify membrane bound P-selectin, soluble P-selectin, P-selectin partial peptides, etc. P-selectin and fragments thereof have the ability to inhibit adhesion to cells, e.g. to inhibit the adhesion of P-selectin to the aforementioned ligands or derivatives thereof fixed to a plastic well (or the adhesion of the aforementioned ligands or derivative thereof to a plastic well to which P-selectin has been fixed). Examples of partial peptides of P-selectin include the peptides described in Published Japanese Translation of a PCT Application H7–501828.

(0013) P-selectin ligand biosynthesis inhibitor signifies inhibitors of glycosyltransferase or the like used in the biosynthesis of the aforementioned ligands of P-selectin. Specific examples include inhibitors of sialyltransferase for transferring sialic acid to oligosaccharide receptors and inhibitors of fucosyltransferase for transferring fucose. Examples include the sialyltransferase inhibitor described in Japanese Unexamined Patent Application Publication H5–247078 and the fucosyltransferase inhibitors described in Chi-Huey Wong et al., J. Am. Chem. Soc. 114, 7321 (1992).

(0014) P-selectin expression inhibitor refers to a substance which inhibits the expression of P-selectin on the cell surface of vascular endothelial cells and the like, such as nitric oxide (NO) donors (Gastroenterology, 107, 1199–1201 (1994); Am. J. Physiol., 267, G562-G568 (1994)). Examples of nitric oxide donors include S-nitrosothiols (Eur. J. Pharm., 144, 379–383 (1987)), N-nitrosothiols (Published Japanese Translation of a PCT Application H5–504760), sydnonimine derivatives (J. Cardiovascular Pharmacology, 18, 522–527 (1991); Biochem. J. 281, 419-424 (1992)), nitroglycerin (J. Pharmacology Experimental Therapeutics, 253, 614–619 (1990)), nitroprussides, etc.

(0015) Examples of the organ transplantation for which the organ transplant rejection suppressant of the present invention can be used includes allotransplantation of organs from human to human, and xenotransplantation of organs from animals such as baboons and pigs to humans. Furthermore, examples of the transplanted organs include heart, lung, liver, kidney, spleen, skin, etc., with heart being a preferable organ. The suppressant of the present invention can be administered parenterally, topically, orally or transcutaneously, and can be administered in various unit dosage forms depending on the method of administration. For example, unit dosage forms suitable for oral administration include powders, tablets, pills, capsules and dragées. Preferably, the suppressant of the present invention is administered intravenously. For intravenous administration, it is used by dissolving or suspending in a pharmaceutically allowable carrier, preferably an aqueous carrier. As the aqueous carrier, for example, water, buffered water, saline or the like can be used. The resultant aqueous solution can be packaged as is, or a freeze-dried preparation can be combined with sterile water before administration. The suppressant of the present invention can include pharmaceutically allowable auxiliaries, such as pH adjusters and buffering agents, tonicity adjusters, lubricants and the like, for instance, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc., as required by the associated physiological condition.

(0016) The suppressant of the present invention is administered to patient before organ transplantation or after organ transplantation in a quantity sufficient to inhibit or at least partially inhibit transplant rejection. The dosage of the suppressant of the present invention varies for instance according to the active ingredient, the method of administration, the severity of the treated disease and the overall health of the patient, but is generally in the range of about 0.5 mg to 1 g of the suppressant of the present invention per day for a patient of 70 kg body weight; preferably, a dosage in the range of about 5 mg to 500 mg of the active ingredient of the present invention is used per day for a patient of 70 kg body weight.

(0017)

(Embodiments) Below, an embodiment is presented to explain the present invention is greater detail; the present invention is however in no way restricted by these embodiments. The fact that P-selectin binding inhibitors are an effective suppressant of rejection in organ transplantation will be explained specifically below on the example of heart transplantation. For the experiment, anti-P-selectin antibody PB1.3 (the antibody of WO93/21956) was used as the P-selectin binding inhibitor.

(0018) Embodiment 1

Effect on rat heart transplant rejection

(Experimental method) Abdominal ectopic heart allotransplantation was performed using F344 male rats as donors and Lewis male rats as recipients. Under a stereoscopic microscope, donor rats under chloral hydrate anesthesia were thoracotomized, the heart was extirpated, and the pulmonary vein and superior and inferior venae cavae of the heart were ligated with silk thread. The extirpated graft heart was stored in an ice-cooled cardioplegic solution until transplanting into the recipient. Next, a mesial incision was made into the abdomen of the recipient rat under chloral hydrate anesthesia, and the abdominal aorta and inferior vena cava were lifted out. After blocking off blood flow by ligating with silk thread and bulldog clamps, a portion of the two blood vessels was cut open, and end-to-side anastomosis of the recipient's abdominal aorta and the aorta of the graft heart and of the recipient's inferior vena cava and the pulmonary artery of the graft heart was performed with 10-0 nylon thread. The ligation with the silk thread was then loosened to restart blood flow to the graft heart, and after confirming the recovery of heartbeat, the abdominal wall was closed. The blood flow cutoff duration for the graft heart was 20 to 30 minutes, and there was normally very little myocardial necrosis due to ischemia. The evaluation of rejection of the graft heart was performed by checking the heartbeat by palpation through the abdominal wall. In the anti-P-selectin antibody treatment group ($n = 5$), PB1.3 was administered intraperitoneally at 400 μg/head 1 day before surgery, on the day of the surgery, and 1, 3, 5 and 7 days after surgery. Furthermore, a non-treatment control group ($n = 4$) was provided as control. Testing for statistical significance of the experimental results was performed by means of a Mann-Whitney U-test.

(Experimental results) The survival times of the graft heart in the non-treatment control group were 7, 9, 10 and 11 days. The survival times of the graft heart in the anti-P-selectin antibody treatment group were 10, 14, 16, 17 and 18 days. The survival time of rat graft heart was significantly ($p < 0.05$) prolonged by administration of anti-P-selectin antibodies.

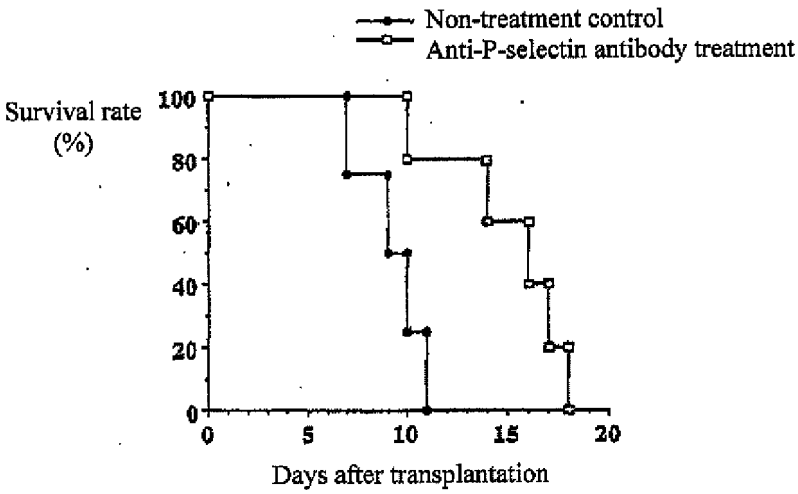
(0019)

(EFFECT OF THE INVENTION) The present invention makes it possible to provide an effective suppressant of rejection in organ transplantation.

(BRIEF DESCRIPTION OF THE DRAWINGS)

(FIGURE 1) Figure 1 shows a heart survival curve for rat graft hearts after anti-P-selectin antibody treatment.

(Figure 1)


Non-treatment control
Anti-P-selectin antibody treatment
Survival rate
(%)
100
80
60
40
20
0
0
5
10
15
20
Days after transplantation

TRANSPERFECT

ALBANY
AMSTERDAM
ATLANTA
AUSTIN
BARCELONA
BOSTON
BRUSSELS
CHARLOTTE
CHICAGO
DALLAS
DENVER
DUBLIN
FRANKFURT
GENEVA
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